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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent Application of:

Joseph A. SORGE et al.

U.S. Application No.: 10/734,563

Filing Date: 12 December 2003

Group Art Unit: 1652

Examiner: R. HUTSON

Confirmation Number: 2401

Title: DNA POLYMERASE COMPOSITIONS FOR QUANTITATIVE PCR AND METHODS THEREOF

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Sir:

APPEAL BRIEF UNDER BOARD RULE 37 C.F.R. § 41.37

In support of the Notice of Appeal filed 30 June 2008, the period for response having been extended by paying the fee for a two-month extension of time in accordance with 37 C.F.R. §1.136(a)(3), and further to Board Rule 41.37, Appellants present this brief and pay the fee of $270.00 (small entity) required under 37 C.F.R. § 41.20(b)(2). With a two-month extension of time, this Appeal Brief is due by 30 October 2008 and is timely filed.

This Appeal responds to the 28 February 2008 final rejection of claims 1-10 and 12-21.

If any additional fees are required or if the submitted payment is insufficient, Appellants request that the required fees be charged to Deposit Account No. 50-3740.

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I. Real Party in Interest

Stratagene, which has been renamed Agilent Technologies Research Corporation, is the real party in interest, as shown by the assignment recorded on 9 April 2004 at reel 014508, frame 0060. Agilent Technologies Research Corporation is a subsidiary of Agilent Technologies, Inc.

II. Related Appeals and Interferences

There are currently no other appeals or interferences, of which Appellants, Appellants' legal representative, or Assignee are aware, that will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. Status of the Claims

Claims 1-26 are pending in this application and are set forth in Appendix I. Claims 1-10 and 12-21 stand finally rejected by the Examiner as noted in the Advisory Action mailed 20 May 2008. Claims 11 and 22-26 are withdrawn from consideration. Appellants appeal the rejection of claims 1-10 and 12-21.

IV. Status of Amendments

Appellants' Amendment After Final filed 28 April 2008 has been entered. *See* Advisory Action mailed 20 May 2008. No subsequent amendments have been filed.

V. Summary of the Claimed Subject Matter

A. Overview of Technology

As discussed in further detail below, the claims at issue in this appeal are generally directed to mutant Archaeal[1] DNA polymerases. DNA polymerases are enzymes that catalyze the synthesis of DNA molecules. Using a preexisting DNA strand as a template, DNA polymerases catalyze the polymerization of nucleotides[2] to form a DNA strand that is complementary to the template DNA. FF[3] 1. Each DNA strand has a direction defined by the orientation of its sugar-phosphate backbone. The end terminating with the 5' carbon is called the 5' end, while the end terminating with the 3' carbon is called the 3' end. DNA polymerases synthesize DNA molecules in the 5' to 3' direction by adding nucleotides to the 3' end of the newly synthesized DNA strand. FF 1.

Some DNA polymerases, including the Archaeal DNA polymerases, also have a 3' to 5' exonuclease activity, sometimes referred to as a "proofreading" activity due to its ability to remove nucleotides that are improperly incorporated into a growing DNA strand. FF 2. Although the 3' to 5' exonuclease activity of Archaeal DNA polymerases helps to enhance the accuracy, or fidelity, of DNA synthesis, there are certain applications where the proofreading activity is not desired. FF 3. For example, DNA polymerases having deficient proofreading

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[1] Together with Eukaryota and Bacteria, the Archaea domain represents one of the three main branches of evolutionary descent. *See* Specification at pages 24-32 for a further discussion of Archaeal DNA polymerases.

[2] In DNA, the four main naturally occurring nucleotides are cytosine, thymine, guanine, adenine. In RNA, uracil replaces thymine.

[3] FF refers to the Findings of Fact set forth in Appendix 4. Although Findings of Facts are not required by the rules in effect at the time this Appeal Brief was filed, Appellants have included them in this paper for the convenience of the Board.

activity are preferred in DNA sequencing reactions to prevent the removal of nucleotide analogs used in the DNA sequencing reactions. FF 4.

The 3' to 5' exonuclease domain in DNA polymerases comprises three conserved motifs (exo I, exo II, and exo III). FF 6. As was known in the art, the 3' to 5' exonuclease activity associated with proofreading DNA polymerases could be reduced or abolished by mutagenesis and, more specifically, by mutating one or more amino acids in one or more of the three conserved motifs, exo I (DXE), exo II (NX_{2-3}(F/Y)D), and exo III (YX_3D) in the 3' to 5' exonuclease domain of DNA polymerases. FF 7-11.

B. V93 Mutation

The application discloses that a wild type Archaeal DNA polymerase or a mutant Archaeal DNA polymerase with deficient 3' to 5' exonuclease activity may be mutated at one or more amino acid positions corresponding to Pro 36, Tyr 37, Ile 38, amino acids 90-97, residues 111-116, and Pro 115 in the wild type *Pyrococcus furiosus* ("Pfu") DNA polymerase (an Archaeal DNA polymerase), and preferably at the valine residue at amino acid position 93 ("V93"). FF 14. Such a mutation can confer reduced uracil base detection to the mutant Archaeal DNA polymerase. FF 15. Wild type Archaeal DNA polymerases stall when they encounter the uracil nucleotide. FF 16. A small percentage of cytosine nucleotides naturally undergo deamination to form uracil. Uracil detection is thought to represent the first step in a pathway to repair DNA cystosine deamination (dCMP → dUMP) in Archaea. FF 17. Although uracil detection may be useful in certain methods, there are other applications where uracil detection is not desired. For example, uracil stalling has been shown to compromise the performance of Archaeal DNA polymerases under standard PCR conditions. FF 18. Therefore,

mutant Archaeal DNA polymerases having a mutation at V93 can be useful, for example, in methods where uracil stalling is not desired. FF 19.

C. Claims

Claims 1-10 and 12-21 are directed to mutant Archaeal DNA polymerases and compositions and kits comprising the same. The mutant Archaeal DNA polymerases 1) comprise at least one mutation in one or more of the conserved exo I, exo II, or exo III domains and a mutation at V93 in one of SEQ ID NOs. 83-108, and 2) are deficient in 3' to 5' exonuclease activity. The amino acid sequences represented by SEQ ID NOs. 83-108 correspond to the wild type amino acid sequences of Archaeal DNA polymerases that were known in the art as of at least the filing date of the present application. FF 20. Claims 1-7 are all independent claims and differ from each other with respect to the recited exo motif(s).

In response to a restriction requirement, Appellants elected with traverse Group I, directed to mutant Archaeal DNA polymerases and compositions and kits comprising said mutant Archaeal DNA polymerases. Response to Restriction Requirement dated 23 October 2006. The Examiner also required restriction to one of the sequences selected from the group of SEQ ID NOs. 83-108. In response, Appellants also elected, with traverse, SEQ ID NO:89, the amino acid sequence corresponding to the wild type Pfu DNA polymerase (FF 23). *Id.*

1. Claim 1 (exo I)

Claim 1 is directed to a mutant Archaeal DNA polymerase deficient in 3' to 5' exonuclease activity and comprising at least one amino acid mutation in the exo I motif and an amino acid mutation at V93 in one of SEQ ID NOs. 83-108. Support for claim 1 can be found throughout the specification, including, for example, at page 1, lines 25-28; page 13, lines 3-16; pages 25-32 (Table II); and Figure 7A.

2. Claim 2 (exo II)

Claim 2 is directed to a mutant Archaeal DNA polymerase deficient in 3' to 5' exonuclease activity and comprising at least one amino acid mutation in the exo II motif and an amino acid mutation at V93 in one of SEQ ID NOs. 83-108. Support for claim 2 can be found throughout the specification, including, for example, at page 2, lines 1-4; page 13, lines 3-16; pages 25-32 (Table II); and Figure 7A.

3. Claim 3 (exo III)

Claim 3 is directed to a mutant Archaeal DNA polymerase deficient in 3' to 5' exonuclease activity and comprising at least one amino acid mutation in the exo III motif and an amino acid mutation at V93 in one of SEQ ID NOs. 83-108. Support for claim 3 can be found throughout the specification, including, for example, at page 2, lines 5-8; page 13, lines 3-16; pages 25-32 (Table II); and Figure 7A.

4. Claim 4 (exo I and exo III)

Claim 4 is directed to a mutant Archaeal DNA polymerase deficient in 3' to 5' exonuclease activity and comprising at least one amino acid mutation in each of the exo I and exo III motifs and an amino acid mutation at V93 in one of SEQ ID NOs. 83-108. Support for claim 4 can be found throughout the specification, including, for example, at page 2, lines 9-12; page 13, lines 3-16; pages 25-32 (Table II); and Figure 7A.

5. Claim 5 (exo II and exo III)

Claim 5 is directed to a mutant Archaeal DNA polymerase deficient in 3' to 5' exonuclease activity and comprising at least one amino acid mutation in each of the exo II and exo III motifs and an amino acid mutation at V93 in one of SEQ ID NOs. 83-108. Support for claim 5 can be found throughout the specification, including, for example, at page 2, lines 13-16; page 13, lines 3-16; pages 25-32 (Table II); and Figure 7A.

6. Claim 6 (exo I and exo II)

Claim 6 is directed to a mutant Archaeal DNA polymerase deficient in 3' to 5' exonuclease activity and comprising at least one amino acid mutation in each of the exo I and exo II motifs and an amino acid mutation at V93 in one of SEQ ID NOs. 83-108. Support for claim 6 can be found throughout the specification, including, for example, at page 2, lines 17-20; page 13, lines 3-16; pages 25-32 (Table II); and Figure 7A.

7. Claim 7 (exo I, exo II, and exo III)

Claim 7 is directed to a mutant Archaeal DNA polymerase deficient in 3' to 5' exonuclease activity and comprising at least one amino acid mutation in each of the exo I, exo II, and exo III motifs and an amino acid mutation at V93 in one of SEQ ID NOs. 83-108. Support for claim 7 can be found throughout the specification, including, for example, at page 2, lines 21-24; page 13, lines 3-16; pages 25-32 (Table II); and Figure 7A.

Claims 8-10 and 12-21 depend directly or indirectly from independent claims 1-7.

VI. Grounds of Rejection

A. Claims 1-10 and 12-21 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement.

B. Claims 1-10 and 12-21 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to enable one of skill in the art to make and use the claimed invention.

C. Claims 1-10 and 12-21 stand provisionally rejected on the grounds of nonstatutory obviousness-type double patenting as allegedly unpatentable over claims 1, 3-5, 13, 15, 17-29, 31-42, 58-66 of copending Application No. 10/298,680.

VII. Argument

A. The Specification Provides Written Description Support for the Claims 1-10 and 12-21

The Examiner rejects claims 1-10 and 12-21 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter that is not described in the specification so as to reasonably convey to a person skilled in the art that Applicants were in possession of the claimed invention at the time the application was filed. Final Office Action at page 4. Appellants respectfully request reversal of this rejection of claims 1-10 and 12-21.[4]

As noted in the MPEP, "the written description requirement for a claimed genus may be satisfied through a sufficient description of a representative number of species by actual reduction to practice (see i)(A), above), reduction to drawings (see i)(B), above), or by disclosure of relevant identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a **known or disclosed** correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus . . . ." MPEP §2163 (emphasis added); *see also*, *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956, 964, 63 USPQ2d 1609, 1613 (Fed. Cir. 2002).

For the reasons discussed below, the specification provides a sufficiently detailed description of the claimed genus by structure and a known and disclosed correlation between structure and function, so as to distinguish it from other mutant Archaeal DNA polymerases, as

[4] Appellants note that the Examiner required election of a single member of the group of SEQ ID NOs. 83-108 recited in claims 1-7. Appellants elected with traverse the wild type Pfu DNA polymerase of SEQ ID NO. 89. The Examiner's rejections under 35 U.S.C. §112, first paragraph for lack of written description and lack of enablement appear applicable to the entire claimed genus and not merely to the elected sequence. Therefore, in response, Appellants address the 112, first paragraph rejections with respect to both the elected species (SEQ ID NO. 89) and the claimed genus (SEQ ID NOs. 83-108).

well as through a description of numerous representative members of the genus, such that one of skill in art would recognize that Appellants were in possession of the claimed invention at the time the application was filed.

1. Representative Species

Claim 1 is directed to a mutant Archaeal DNA polymerase comprising: at least one amino acid mutation in the exo I motif, and an amino acid mutation at V93 in an amino acid sequence selected from one of SEQ ID NOs. 83-108, where said mutant Archaeal DNA polymerase is deficient in 3' to 5' exonuclease activity. Claims 2-7 are similarly directed to mutant Archaeal DNA polymerases with an amino acid mutation at V93 in an amino acid sequence selected from one of SEQ ID NOs. 83-108 and deficient in 3' to 5' exonuclease activity but further recite at least one amino acid mutation in the exo II motif (claim 2), the exo III motif (claim 3), each of the exo I and exo III motifs (claim 4), each of the exo II and exo III motifs (claim 5), each of the exo I and exo II motifs (claim 6), and each of the exo I, exo II, and exo III (claims 7).

The amino acid sequences represented by SEQ ID NOs. 83-108 correspond to the wild type amino acid sequences of Archaeal DNA polymerases that were known in the art as of at least the filing date of the present application. FF 20. In the elected invention, the mutant Archaeal DNA polymerase comprises the recited mutations in the amino acid of SEQ ID NO. 89, which corresponds to the known, wild type amino acid sequence of *Pyrococcus furiosus* ("Pfu") DNA polymerase. FF 23.

Archaeal DNA polymerases, like Pfu DNA polymerase, naturally possess 3' to 5' exonuclease (proofreading) activity. FF 5. It was known in the art that the 3' to 5' exonuclease domain in DNA polymerases comprises three conserved motifs (exo I, exo II, and exo III). FF 6.

The exo I motif is represented by the consensus amino acid sequence DXE. FF 8. The exo II motif is represented by the consensus amino acid sequence $NX_{2-3}(F/Y)D$. FF 9. The exo III motif is represented by the consensus amino acid sequence YX_3D. FF 10.

It was also known in the art that DNA polymerases with 3' to 5' exonuclease activity, like Archaeal DNA polymerases, could be mutated in the conserved exo I, exo II, or exo III motifs to generate mutant DNA polymerases having reduced or abolished 3' to 5' exonuclease activity. FF 11. Thus, there was a known correlation in the art between the conserved exo I, exo II, and exo III motifs of DNA polymerases and 3' to 5' exonuclease activity. FF 12. This known correlation between structure and function is not disputed by the Examiner. FF 13.

a) Elected Invention (SEQ ID NO:89)

As for the elected invention, the specification discloses several examples of mutant Pfu DNA polymerases comprising a mutation at V93. FF 28. The specification also discloses specific examples of mutant Archaeal DNA polymerases with deficient 3' to 5' exonuclease activity, including a *Thermococcus sp.* (JDF-3) DNA polymerase with a mutation at the position corresponding to D141 and/or E143 in the conserved exo I motif. FF 29. The specification also teaches that one skilled in the art would be able to make other mutant Archaeal DNA polymerases, such as a mutant Pfu DNA polymerase, with deficient 3' to 5' exonuclease activity by mutating one or more amino acid within the conserved exo I, II, and III motifs. FF 30. Indeed, the specification discloses an example of triple mutant Pfu DNA polymerases comprising a mutation at V93 (V93R or V93E) and two mutations in the conserved exo I motif (D141A and E143A), where the mutant Pfu DNA polymerase possesses deficient 3' to 5' exonuclease activity. FF 32.

b) Non-Elected Invention (SEQ ID NOs. 83-88 and 90-108)

As for the recited wild type amino acid sequences other than Pfu, the specification discloses numerous examples of Archaeal DNA polymerases comprising a mutation at V93, including *Pyrococcus sp.* (Deep Vent)[5], *Thermococcus gorgonarius* (Tgo)[6], *Pyrococcus sp.* (KOD)[7], *Thermococcus litoralis* (Vent)[8], and *Thermococcus sp.* (JDF-3)[9]. FF 28. In addition, the specification teaches in detail how to make other mutant Archaeal DNA polymerases using well known methods. FF 37-38. Given this guidance, one of skill in the art would be able to generate other mutant Archaeal DNA polymerases, such as mutant versions of SEQ ID NOs. 84-87, 91, and 94-108, having a mutation at V93.

The specification also discloses specific examples of mutant Archaeal DNA polymerases with deficient 3' to 5' exonuclease activity, including a *Thermococcus sp.* (JDF-3) DNA polymerase with a mutation at the position corresponding to D141 and/or E143 in the conserved exo I motif. FF 29. The specification also teaches that one skilled in the art would be able to make other mutant Archaeal DNA polymerases, such as mutant versions of SEQ ID NOs. 83-88 and 90-108, with deficient 3' to 5' exonuclease activity by mutating one or more amino acids within the conserved exo I, II, and III motifs. FF 30. The specification also discloses a prophetic example teaching how to make mutant Archaeal DNA polymerases (Tgo, KOD, or JDF-3) comprising a mutation at V93 and at the conserved D141 and E143 residues of the exo I motif and having deficient 3' to 5' exonuclease activity. FF 31.

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5 Corresponds to the wild type sequence SEQ ID NO. 88 in claims 1-7. FF 22.

6 Corresponds to the wild type sequence SEQ ID NO. 93 in claims 1-7. FF 26.

7 Corresponds to the wild type sequence SEQ ID NO. 92 in claims 1-7. FF 25.

8 Corresponds to the wild type sequence SEQ ID NO. 83 in claims 1-7. FF 21.

9 Corresponds to the wild type sequence SEQ ID NO. 90 in claims 1-7. FF 24.

The Examiner asserts that Appellants' "representative species are not sufficient in describing the breadth of the claimed genus." Advisory Action at page 2. The Examiner, however, provides no evidence or reasoning to support this conclusion. In so doing, the Examiner overlooks both the level of skill and knowledge in the art. "What constitutes a 'representative number' is an inverse function of the skill and knowledge in the art." MPEP §2163. Here, the level of skill in the art is high. FF 35. The Examiner does not contest this. FF 36.

Furthermore, "[s]atisfactory disclosure of a 'representative number' depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed." MPEP §2163. For the elected invention, one of skill in the art would recognize the common features as a mutation at V93 and at least one mutation in one or more of the conserved exo I, exo II, or exo III motifs in the wild type Pfu DNA polymerase (SEQ ID NO. 89) and deficient 3' to 5' exonuclease activity. For the non-elected invention, one of skill in the art would similarly recognize the common features of the claimed genus as a mutation at V93 and at least one mutation in one or more of the conserved exo I, exo II, or exo III motifs in the wild type amino acid sequence of an Archaeal DNA polymerase corresponding to one of SEQ ID NOs. 83-88 and 90-108 and deficient 3' to 5' exonuclease activity

The evidence shows that Appellants were in possession of the common features of not only the elected invention, but also of the full scope of the claimed genus. Specifically, Appellants discovered the mutation at V93 and produced V93 mutants in several Archaeal DNA polymerase species. FF 28. In addition, there was a disclosed and known correlation between the conserved exo I, exo II, and exo III motifs and 3' to 5' exonuclease activity. FF 12.

Furthermore, the sequences of the wild type Archaeal DNA polymerases recited in the claims (SEQ ID NOs. 83-108) were known. FF 20. Thus, given the high level of skill in the art, the knowledge in the art, and Appellants' disclosure, one of skill in the art would have recognized that Appellants possessed the common features of the claimed genus and thus adequately described the same.

Initially, the Examiner asserted that "[t]he specification . . . only provides those Archaeal DNA polymerases wherein said mutant DNA polymerase has the amino acid sequence of SEQ ID NO:89 with a single mutation at position 93 . . . wherein said DNA polymerase is deficient in 3'-5' exonuclease activity." Non-Final Office Action mailed 31 May 2007. Appellants responded by pointing out that the specification discloses additional mutant polymerase beyond the single one identified by the Examiner, including V93 mutants in other Archaeal DNA polymerases, and teaches that the V93 mutant Archaeal DNA polymerases can be further modified to include at least one mutation in one or more of the conserved exo I, exo II, or exo III motifs in the 3' to 5' exonuclease domain.

In response, the Examiner acknowledged that "applicants provide additional mutations of the disclosed polymerases that are deficient in 3'-5'exonuclease activity . . . ." Nevertheless, the Examiner merely concludes, without any evidence or reasoning, that "these additional referred to mutants are not sufficient to adequately describe the claimed genus of **any** archael [*sic*, archaeal] DNA polymerase deficient in 3'-5' exonuclease activity, wherein said mutant comprises at least one mutation in a exoI, exoII, or exoIII motif and another mutation at position V93 of the polymerase." Final Office Action at pages 5-6 (emphasis added in bold).

The Examiner also asserts:

> Applicants [*sic*, Applicants'] argument that the disclosed species of archael [*sic*, archaeal] DNA polymerases and the disclosed mutants of

> SEQ ID NO: 89 are sufficient to provide a particular structure to function/activity relationship that would put one in possession of the genus of **all possible mutant archael DNA polymerases** with a reduced base analog detection [*sic*, deficient 3' to 5' exonuclease] activity comparing [*sic*, comprising] a mutation in a exoI, exoII, or exoIII motif and having a mutation corresponding to V93 is not persuasive.

Id. (emphasis added in bold). As the Examiner has not provided any evidence or reasoning why the disclosed mutant Archaeal DNA polymerases are not descriptive of either the elected invention or the full scope of the claimed invention, he has failed to establish a *prima facie* case of lack of written description, and the rejection should be reversed on this ground alone.

Moreover, contrary to the Examiner's position, the claims are not directed to "any" mutant Archaeal DNA polymerase or "all possible mutant archael DNA polymerases." Rather, the claims are directed to mutant Archaeal DNA polymerases deficient in 3' to 5' exonuclease activity and comprising the recited mutations "in an amino acid sequence selected from one of SEQ ID NOs. 83-108." And the elected invention recites that the mutant DNA polymerase comprises the recited mutations in the amino acid sequence of SEQ ID NO. 89. Thus, in maintaining this written description rejection, the Examiner fails to consider recited elements of the claimed invention. As such, the Examiner's written description rejection is based on an erroneous construction of the claims and can be reversed on this ground alone.

2. Disclosure of Relevant Identifying Characteristics

In addition to describing a sufficient number of representative species, the written description requirement for a claimed genus may satisfied through disclosure of relevant identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or some combination of characteristics. *See* MPEP §2163; *see also*, *Enzo Biochem,*

323 F.3d at 964, 63 USPQ2d at 1613. Here, the written description requirement for both the elected invention and the full scope of the claimed genus is satisfied through disclosure of structure and functional characteristics coupled with a known and disclosed correlation between function and structure.

As to function, claims 1-7 recite that the mutant Archaeal DNA polymerase is deficient in 3' to 5' exonuclease activity. Methods for assaying DNA polymerases for 3' to 5' exonuclease activity were known in the art and disclosed in the specification. FF 39. The Examiner does not contest that one of skill in the art would be able to test for the activity. FF 40.

As to structure, claims 1-7 are drawn to a mutant Archaeal DNA polymerase comprising at least one mutation in one or more of the conserved exo I, exo II, or exo III motifs and a mutation at V93 in one of the wild type Archaeal DNA polymerases of SEQ ID NOs. 83-108, or in the case of the elected invention, SEQ ID NO. 89. Thus, claims 1-7 set forth complete or partial structure, *i.e.*, SEQ ID NOs. 83-108 comprising the recited mutations, coupled with a known and disclosed correlation between function, *i.e.*, deficient in 3' to 5' exonuclease activity and structure (*i.e.*, at least one mutation in one or more of the conserved exo I, exo II, or exo III motifs). The specification also discloses representative species, as discussed above.

The Examiner asserts that "the specification fails to describe additional representative species of these mutant DNA polymerases by any identifying structural characteristics or properties other than the activities recited in the claims, for which no predictability of structure is apparent." Final Office Action at page 6; Advisory Action at page 2. Appellants' response is two-fold.

First, the claimed mutant polymerases are clearly defined by characteristics "other than the activities recited in the claims." For example, in addition to reciting that the mutant Archaeal

DNA polymerases are deficient in 3' to 5' exonuclease activity, claims 1-7 also recite that the mutant Archaeal DNA polymerase comprises an amino acid mutation at V93 and at least one amino acid mutation in the recited 3' to 5' exonuclease motifs in an amino acid sequence selected from one of SEQ ID NOs. 83-108, or in the case of the elected invention, SEQ ID NO. 89.

Second, contrary to the Examiner's unsupported assertions, there is a predictable structure for the recited function. As discussed in the specification, there was a known correlation in the art between the conserved exo I, exo II, and exo III motifs and 3' to 5' exonuclease activity. FF 12. Given this known and disclosed correlation between structure and function, the evidence indicates that one of skill in the art would recognize a predictable relationship between the recited structure (at least one mutation in one or more of the conserved exo I, exo II, or exo III motifs of one of SEQ ID NOs. 83-108, or in the case of the elected species, SEQ ID NO. 89) and the recited function (deficient in 3' to 5' exonuclease activity). This evidence stands unrebutted.

In response to Appellants' statements about these other characteristics recited in the claims, the Examiner argues, for the first time in the Advisory Action, that the claim language "at least one amino acid mutation . . ." somehow eliminates the recited characteristics and, thus, allegedly renders the predictable structure for the recited function insufficient for purposes of satisfying the written description requirement. Specifically, the Examiner asserts:

> Applicants [*sic*, Applicants'] statements that the claims contain "characteristics" which are they [*sic*] are directed to an Archael [*sic*, Archaeal] DNA polymerase comprising certain mutations continues to be acknowledged, however, given applicants [*sic*, applicants'] use of the terminology "at least one amino acid mutation ..." in the claim, [*sic*, are] not found persuasive. This language effectively eliminates applicant's recited "characteristics" thus opening up the claimed genus.

> Thus the predictable structure for the recited function for the encompassed polymerases of the claimed genus is insufficient.

Advisory Action at page 2. The Examiner also asserts that "[w]hile Applicants argue that they do describe characteristics of the claimed genus, these 'characteristics' are not limitations of the breadth of the claimed genus." *Id.*

Appellants' response is that the Examiner's claim construction is erroneous. During prosecution, the Office must give claims their broadest reasonable construction, consistent with the specification. *See* MPEP § 2111. Appellants submit that construing the "at least one mutation . . ." language in claims 1-7 as eliminating the other recited elements of the claims is neither reasonable nor consistent with the specification and improperly reads out of the claims other express recitations.

The claim language "at least one mutation . . ." does not alter the known and disclosed correlation between the conserved exo I, exo II, and exo III motifs (structure) and the 3' to 5' exonuclease activity (function). Nor does it change how this predictable relationship between structure and function should be used in analyzing compliance with the written description requirement.

The language at issue is part of the recitation relating to the mutations in the conserved 3' to 5' exonuclease motifs. As discussed above, claims 1-7 recite that the mutant Archaeal DNA polymerase comprises, *inter alia*, "at least one mutation in" one or more of the conserved exo I, exo II, or exo III motifs. Notably, the exo I (DXE), exo II ($NX_{2-3}(F/Y)D$), and exo III (YX_3D) motifs span a limited number of amino acids. FF 8-10. Thus, Appellants do not understand how the recitation of "at least one mutation" within one or more of these 3 exonuclease motifs "effectively eliminates applicant's recited 'characteristics[.]'"

The "at least one mutation . . ." language of the claims merely conveys that there may be

one or more mutations within the exo I, exo II, and/or exo III motifs, as exemplified in Appellants' mutant Archaeal DNA polymerases comprising two mutations within the exo I motif. FF 31-32. The language does not have any bearing on the mutation at position V93. Nor does it change the recitation that the mutant Archaeal DNA polymerase is "deficient in 3' to 5' exonuclease activity," or that it comprises the recited mutations "in an amino acid selected from one of SEQ ID NOs. 83-108," or in the case of the elected invention, SEQ ID NO. 89. Accordingly, the Examiner's erroneous claim construction, on its own, provides a basis for reversal of the written description rejection.

Moreover, given 1) the known wild type Archaeal DNA polymerase sequences recited in the claims (*i.e.*, SEQ ID NOs. 83-108, or in the case of the elected invention, SEQ ID NO. 89); 2) Appellants' disclosure of an amino acid mutation at V93; and 3) the known and disclosed correlation between the conserved, DNA polymerase exo I, exo II, and exo III motifs (structure) and 3' to 5' exonuclease activity (function), Appellants have adequately described the claimed subject matter. Therefore, under a proper claim construction, the claims recite sufficient structure and function to show that Appellants were in possession of not only the elected species but also the full scope of the claimed genus.

Accordingly, for the reasons discussed above, Appellants respectfully request reversal of the written description rejection of claims 1-10 and 12-21 under 35 U.S.C. § 112, first paragraph.

B. Claims 1-10 and 12-21 Are Enabled

The Examiner rejects claims 1-10 and 12-21 under 35 U.S.C. §112, first paragraph, alleging that the specification does not enable one of skill in the art to make and use the invention commensurate in scope with the claimed invention. Final Office Action at page 7. Appellants respectfully request reversal of this rejection of claims 1-10 and 12-21.

The Examiner draws unsupported conclusions that the presently claimed invention lacks enablement for the full scope of the claims. Appellants will now provide a full analysis of the issue and show that the conclusions of the Examiner are unsupportable under a *Wands* analysis. Each relevant *Wands* factor is addressed below.

1. The Breadth of the Claims

Independent claims 1-7 are directed to mutant Archaeal DNA polymerases comprising at least one amino acid mutation in one or more of the conserved exo I, exo II, or exo III motifs and an amino acid mutation at V93 in an amino acid sequence selected from one of SEQ ID NOs. 83-108, where the Archaeal DNA polymerase is deficient in 3' to 5' exonuclease activity. The sequences represented by SEQ ID NOs. 83-108 correspond to the known amino acid sequences of wild type Archaeal DNA polymerases. FF 20. In the elected invention, the mutant DNA polymerase comprises the recited mutations in SEQ ID NO. 89, the wild type Pfu DNA polymerase sequence. FF 23. Thus, the claimed mutants comprise the recited mutations in one of several known wild type Archaeal DNA polymerase sequences and are fully defined with regard to structural identity.

According to the Examiner, the claims cover an infinite number of mutant Archaeal DNA polymerases. Specifically, the Examiner asserts that

> producing variants as claimed by applicants (i.e., deficient in 3'-5' exonuclease activity) requires that one of ordinary skill in the art know or be provided with guidance for the selection of which of the **infinite number of mutant archael [*sic*, archaeal] polymerases** would have the claimed property. Without such guidance one of ordinary skill would be reduced to the necessity of producing and testing **all of the virtually infinite possibilities**. This would clearly constitute undue experimentation. For example applicants state in their specification that encompassed by "archael" [*sic*, "archaeal"] DNA polymerases are both the Family B/pol I-type group or the pol II group, yet it appears that applicants [*sic*, applicants'] arguments are predominantly in support of the Family B/pol II group.

Final Office Action at page 8 (emphasis added).

Appellants do not understand the Examiner's reference to the "infinite number of mutant archaeal polymerases," however it appears this statement may be tied to the Examiner's erroneous claim construction discussed above in the written description section, and in particular to the Examiner's position that the claims cover "all possible mutant archaeal DNA polymerases." For the reasons discussed above, Appellants assert that such a claim construction is erroneous.

Furthermore, Appellants do not understand the Examiner's reference to the "Family B/pol II group" or the reasoning relied on to support the assertions regarding the same. The specification refers to Family B/pol I and pol II polymerases but not Family B/pol II polymerases. FF 27. Furthermore, any distinction between Family B/pol I and pol II polymerases overlooks the recitation in the claims that the Archaeal DNA polymerase comprises the recited mutations in an amino acid sequence selected from SEQ ID NOs. 83-108, or in the case of the elected invention, SEQ ID NO. 89. Notably, all of the recited sequences (SEQ ID NOs 83-108) are Family B/pol I type DNA polymerases.

As with the written description rejection, for the first time in the Advisory Action, the Examiner also takes issue with the "at least one mutation . . ." language in claims 1-7, as it relates to the enablement rejection. Specifically, the Examiner states:

> As above applicants [*sic*, applicants'] comments regarding "characteristics" of the claimed genus are acknowledged, however it continues that these characteristics are not descriptive of the breadth of the claimed genus. Much of the problems with the current [enablement] rejection stems from the same issue as above, the breadth of the claimed genus that stems from applicants [*sic*, applicants'] use of the terminology "DNA polymerase comprising at least one mutation in the . . . ."

Advisory Action at page 2.

For the reasons discussed above, however, Appellants submit that construing the "at least one mutation . . ." language in claims 1-7 as eliminating the other recited elements of the claims is erroneous and improper and warrants reversal of the Examiner's rejection.

2. The Nature of the Invention

Appellants have discovered that a mutation at V93 within Archaeal DNA polymerases alters certain characteristics of the polymerases, such as uracil detection. FF 14-15. Appellants have also discovered that the V93 mutation can be combined with other mutations, such as one or more mutations within one or more of the conserved exo I, exo II, or exo III motifs in the 3' to 5' exonuclease domain of DNA polymerases. As discussed in the specification, the 3' to 5' exonuclease activity of a DNA polymerase can be reduced or abolished by mutating one more of the exo I, exo II, or exo III motifs. FF 7-11.

3. The State of the Art

At the time of filing of this application, the art was well developed, both from the standpoint of mutagenesis schemes and from the standpoint of knowledge of Archaeal DNA polymerase structure and function. Indeed, the application cites numerous Archaeal DNA polymerases that, at the time of filing, had been sequenced and their respective sequences published, including the sequences recited in claims 1-7 (*i.e.*, SEQ ID NOs. 83-108). FF 20. Likewise, those of skill in the art were, at the time of filing, well aware of numerous methods for making and screening mutants of many different proteins. FF 37-40.

4. The Level of Skill of Those of Skill in the Art

As indicated repeatedly by the Federal Circuit, the level of skill in the art of biotechnology is exceptionally high. The Examiner does not dispute this finding. FF 36.

5. The Level of Predictability in the Art

The Federal Circuit has asserted that biotechnology, at least at the time of filing of the present application, was an unpredictable art requiring an elevated level of disclosure for enablement. Appellants submit that the present application has satisfied that heightened level of disclosure by providing numerous examples of Archaeal DNA polymerase mutants having a mutation at V93, each of which can be used by those of skill in the art as guides for developing additional mutants according to the claims. FF 28. As taught in the specification, it was known in the art that DNA polymerases with 3' to 5' exonuclease activity could be mutated in the conserved exo I, exo II, or exo III motifs to generate mutant DNA polymerases having reduced or abolished 3' to 5' exonuclease activity. FF 11 and 29-30. The specification also discloses examples teaching how to make mutant Archaeal DNA polymerases comprising a V93 mutation and at least one mutation in one of the conserved exonuclease motifs. FF 31-33. Thus, although the art in general may be defined by the Federal Circuit as being unpredictable, the specific mutations recited in the claims are fully described. Furthermore, the functional effects of mutations within the conserved exo I, exo II, and/or exo III motifs have been determined, and one of skill in the art would be able to predict with reasonable confidence that additional mutations within these defined residues would have similar effects.

Regarding the level of predictability in the art, the Examiner asserts:

> The specification does not establish: (A) regions of the protein structure which may be modified without effecting [*sic*, affecting] deficiencies in 3'-5' exonuclease activity; (B) the general tolerance of Archael [*sic*, Archaeal] Family B/pol I-type and pol II-type DNA polymerase to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residue of any Archael [*sic*, Archaeal] DNA polymerase including both Family B/pol I-type and pol II-type DNA polymerases with an expectation of obtaining the desired biological function . . . .

Final Office Action at page 9.

In response to points (A)-(C), as discussed above, the specification, coupled with the knowledge in the art, provides substantial guidance as to the specific, conserved motifs within Archaeal DNA polymerases (including the elected invention (SEQ ID NO. 89) and the full scope of the claimed genus (SEQ ID NOs. 83-108)) that are associated with the 3' to 5' exonuclease activity of the polymerases. As explained in the specification:

> The 3'-5' exonuclease activity associated with proofreading DNA polymerases can be reduced or abolished by mutagenesis. Sequence comparisons have identified three conserved domains (exo I (DXE), II (NX_{2-3}(F/Y)D), III (YX_3D) in the 3'-5' exonuclease domain of DNA polymerases (reviewed V. Derbyshire, J.K. Pinsonneault, and C.M. Joyce, Methods Enzymol. 262, 363 (1995)).

FF 7. Thus, contrary to the Examiner's assertions, the specification establishes that it was known in the art that specific, conserved domains are associated with 3' to 5' exonuclease activity in DNA polymerases and that mutations in those domains result in diminished 3' to 5' exonuclease activity. Moreover, given the known correlation between structure and function, the specification provides a rational and predictable scheme for modifying amino acids in the conserved exo I, exo II, and/or exo III domains of an Archaeal DNA polymerase to generate mutant DNA polymerases having deficient 3' to 5' exonuclease activity, as recited in the claims. The Examiner provides no evidence or reasoning to doubt these teachings in the specification.

6. The Amount of Direction Provided by Appellants and the Existence of Working Examples

The claimed invention is clearly and fully described in the application, including not only specific mutations literally covered by the claims, but methods for making such mutations and methods of screening such mutations for expected activity. Even the Examiner acknowledges that "methods to produce specific variants (*i.e.*, exo I, exoII, exoIII and mutants of V93) of a

known sequence such as site specific mutagenesis, random mutagenesis, etc. are well known to the skilled artisan . . . ." FF 38.

Furthermore, the specification provides numerous examples of Archaeal DNA polymerases having a mutation at V93, including a mutant Pfu DNA polymerase. FF 28. Given the guidance in the specification and the well known mutagenesis methods, one of skill in the art would be able to generate other mutant Archaeal DNA polymerases having a mutation at V93. FF 37-38.

The specification also provides specific examples of mutant Archaeal DNA polymerases with deficient 3' to 5' exonuclease activity, including those with a mutation at the position corresponding to D141 and/or E143 in the conserved 3' to 5' exonuclease domain. FF 29. The specification also teaches that one skilled in the art would be able to make other mutant Archaeal DNA polymerases, such as a mutant Pfu DNA polymerase, with deficient 3' to 5' exonuclease activity by mutating one or more amino acids within the conserved exo I, II, and III motifs. FF 30. Furthermore, it is not contested that methods for assaying DNA polymerases for 3' to 5' exonuclease activity were known in the art and disclosed in the specification. FF 39-40. Finally, Appellants combined the 3' to 5' exonuclease mutants with the V93 mutants to produce mutant Archaeal DNA polymerases with deficient 3' to 5' exonuclease activity. FF 31-33. The Examiner does not provide any evidence or reasoning why the specification does not enable those disclosed mutant polymerases. FF 34.

The Examiner asserts that "(D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful." Final Office Action at page 9. Appellants' response is that by reciting that the Archaeal polymerase comprises at least one mutation in one or more of the exo I, exo II or exo III domains, the claims do provide

significant guidance as to which mutant polymerases will have the desired activity, *i.e.*, deficient 3' to 5' exonuclease activity. Furthermore, methods for making the claimed mutant polymerases and screening for the recited activity were known in the art and disclosed in the specification. FF 37-40. Given the limited number of amino acid residues falling within the conserved 3' to 5' exonuclease domains recited in the claims and the known correlation between structure and function (FF 7-13), one of skill in the art could readily make and screen DNA polymerases other than those disclosed in the application to determine if they possess the desired activity. Thus the specification demonstrates with reasonable specificity and predictability how to make and use other potential embodiments across the full scope of the claims.

7. The Quantity of Experimentation Needed to Make or Use the Claimed Invention

The Federal Circuit has made clear that the quantity of experimentation needed to make or use an invention is not dispositive of enablement. Moreover, the test for undue experimentation is not merely quantitative, because a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed. *Ex parte Jackson*, 217 USPQ 804, 807 (CCPA 1982); *see also*, *Johns Hopkins Univ. v. Cellpro, Inc.*, 152 F.3d 1342, 1360, 47 USPQ2d 1705, 1719 (Fed. Cir. 1989 ("test [for undue experimentation] is not merely quantitative . . . if it is merely routine.").

In the present situation, the number of mutants literally encompassed by the present claims is finite and defined by structure. Although many possible mutants are covered by the claims, it would be a matter of mere routine experimentation, much of which could be performed

using automated screening assays, to distinguish between mutant DNA polymerases having the desired function and those that do not and, thus, fall outside the scope of the claims.

8. Summary

The Examiner's erroneous claim construction, on its own, or in combination with the unsupported assertions regarding the level of predictability and lack of guidance in the specification warrant a reversal of the Examiner's enablement rejection. Moreover, Appellants submit that all of the *Wands* factors either weigh heavily toward enablement of both the elected invention (SEQ ID NO. 89) and the full scope of claims 1-7 or are inconclusive in the determination: no factor weighs against full enablement of the claimed invention. Therefore, for the additional reason that the preponderance of the factors weigh in favor of enablement, Appellants respectfully request reversal of the enablement rejection of claims 1-10 and 12-21 under 35 U.S.C. § 112, first paragraph.

C. Double Patenting Rejection

The Examiner provisionally rejects claims 1-10 and 12-21 on the grounds of nonstatutory obviousness-type double patenting as allegedly unpatentable over claims 1, 3-5, 13, 15, 17-29, 31-42, 58-66 of copending Application No. 10/298,680. Final Office Action at pages 10-11. Appellants request that the Office hold this provisional rejection in abeyance until one of the two patent applications in question is deemed to be in condition for allowance. At that time, if the Office still believes that the claims conflict with each other, Appellants will take the appropriate action to address the possibility of double patenting. *See* MPEP §804.

CONCLUSION

For the reasons given above, pending claims 1-10 and 12-21 are allowable and reversal of the Examiner's rejection is respectfully requested.

To the extent any extension of time under 37 C.F.R. § 1.136, not accounted for above, is required to obtain entry of this Appeal Brief, such extension is hereby respectfully requested. If there are any fees due that are not enclosed, including any fees required for an extension of time under 37 C.F.R. § 1.136, please charge such fees to Deposit Account No. 50-3740.

Respectfully submitted,
Joseph A. SORGE et al.

Date: 30 October 2008

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APPENDIX 1
CLAIMS ON APPEAL

1. (Rejected) An Archaeal DNA polymerase comprising at least one amino acid mutation in the exoI motif and an amino acid mutation at V93 in an amino acid sequence selected from one of SEQ ID NOs. 83-108, wherein said Archaeal DNA polymerase is deficient in 3'-5' exonuclease activity.

2. (Rejected) An Archaeal DNA polymerase comprising at least one amino acid mutation in the exoII motif and an amino acid mutation at V93 in an amino acid sequence selected from one of SEQ ID NOs. 83-108, wherein said Archaeal DNA polymerase is deficient in 3'-5' exonuclease activity.

3. (Rejected) An Archaeal DNA polymerase comprising at least one amino acid mutation in the exo III motif and an amino acid mutation at V93 in an amino acid sequence selected from one of SEQ ID NOs. 83-108, wherein said Archaeal DNA polymerase is deficient in 3'-5' exonuclease activity.

4. (Rejected) An Archaeal DNA polymerase comprising at least one amino acid mutation in each of the exo I and exo III motifs and an amino acid mutation at V93 in an amino acid sequence selected from one of SEQ ID NOs. 83-108, wherein said Archaeal DNA polymerase is deficient in 3'-5' exonuclease activity.

5. (Rejected) An Archaeal DNA polymerase comprising at least one amino acid mutation in each of the exo II and exo III motifs and an amino acid mutation at V93 in an amino acid sequence selected from one of SEQ ID NOs. 83-108, wherein said Archaeal DNA polymerase is deficient in 3'-5' exonuclease activity.

6. (Rejected) An Archaeal DNA polymerase comprising at least one amino acid mutation in each of the exo I and exoII motifs and an amino acid mutation at V93 in an amino acid sequence selected from one of SEQ ID NOs. 83-108, wherein said Archaeal DNA polymerase is deficient in 3'-5' exonuclease activity.

7. (Rejected) An Archaeal DNA polymerase comprising at least one amino acid mutation in each of the exoI, exo II, and exoIII motifs and an amino acid mutation at V93, in an amino acid sequence selected from one of SEQ ID NOs. 83-108,wherein said Archaeal DNA polymerase is deficient in 3'-5' exonuclease activity.

8. (Rejected) The mutant Archaeal DNA polymerase of any of claims 1-7, wherein said mutant Archaeal DNA polymerase is selected from the group consisting of: KOD, Pfu, and JDF-3 DNA polymerase.

9. (Rejected) The mutant Archaeal DNA polymerase of any of claims 1-7, wherein said mutation at position V93, is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a Valine to Aspartic acid substitution, a Valine to Glutamine substitution, or a Valine to Asparagine substitution.

10. (Rejected) The mutant Archaeal DNA polymerase of any of claims 1-7, wherein said mutation in exo I motif is selected from the group consisting of: aspartic acid (D) to threonine (T), aspartic acid (D) to alanine (A) and glutamic acid (E) to alanine (A).

11. (Withdrawn) An isolated polynucleotide comprising a nucleotide sequence encoding a mutant Archaeal DNA polymerase of any of claims 1-7.

12. (Rejected) A composition comprising a mutant Archaeal DNA polymerase of any of claims 1-7.

13. (Rejected) The composition of claim 12, further comprising an enzyme with reverse transcriptase activity.

14. (Rejected) The composition of claim 13, wherein said enzyme with reverse transcriptase is a second mutant DNA polymerase.

15. (Rejected) The composition of claim 13, wherein said enzyme with reverse transcriptase is the mutant Archaeal DNA polymerase which contains an increased reverse transcriptase activity.

16. (Rejected) The composition of claim 12, further comprising a PCR additive.

17. (Rejected) A kit comprising a mutant Archaeal DNA polymerase of any of claims 1-7 and packaging material therefor.

18. (Rejected) The kit of claim 17, further comprising an enzyme with reverse transcriptase activity.

19. (Rejected) The kit of claim 18, wherein said enzyme with reverse transcriptase is a second mutant DNA polymerase.

20. (Rejected) The kit of claim 18, wherein said enzyme with reverse transcriptase is the mutant Archaeal DNA polymerase which contains an increased reverse transcriptase activity.

21. (Rejected) The kit of claim 17, further comprising a PCR additive.

22. (Withdrawn) A method for DNA synthesis comprising:

(a) providing a mutant Archaeal DNA polymerase of any of claims 1-7; and

(b) contacting said mutant Archaeal DNA polymerase with a polynucleotide template to permit DNA synthesis.

23. (Withdrawn) A method for determining the abundance of a polynucleotide template, comprising

(a) providing a mutant Archaeal DNA polymerase of any of claims 1-7;

(b) contacting said mutant Archaeal DNA polymerase with said polynucleotide template to produce amplified product; and

(c) determining the abundance of said amplified product, wherein said abundance of said amplified product is indicative of the abundance of said polynucleotide template.

24. (Withdrawn) The method of claim 23, wherein said polynucleotide template is a RNA molecule, and wherein said RNA molecule is reverse transcribed into cDNA before the contacting step (b).

25. (Withdrawn) The method of claim 24, wherein said RNA is reverse transcribed by an enzyme with reverse transcriptase activity.

26. (Withdrawn) The method of claim 25, wherein said RNA is reverse transcribed by said mutant Archaeal DNA polymerase which also contains an increased reverse transcriptase activity.

APPENDIX 2
EVIDENCE SUPPORTING APPEAL BRIEF

Other than the specification, Appellants do not rely on any additional evidence in support of this appeal brief.

APPENDIX 3
DECISIONS IN PROCEEDINGS RELATED TO APPEAL

There are no decisions in proceedings related to this appeal.

APPENDIX 4
STATEMENT OF FACTS

1. "DNA polymerases synthesize DNA molecules in the 5' to 3' direction from deoxynucleoside triphosphates (nucleotides) using a complementary template DNA strand and a primer by successively adding nucleotides to the free 3'-hydroxyl group of the growing strand." Specification at page 1, lines 16-19.

2. In addition to DNA synthesis activity, Archaeal DNA polymerases have a 3' to 5' exonuclease activity, sometimes referred to as a "proofreading" activity due to its ability to excise nucleotides that are improperly incorporated into a growing polynucleotide strand. Specification at 1, lines 22-23.

3. As explained in the specification:

> DNA synthesis activity acts to polymerize nucleotides while 3'-5' exonuclease has an editing or proof-reading function to enhance the fidelity of the synthesis. Thus highly efficient DNA synthesis is generally achieved at the expense of high fidelity and vice versa. The 3'-to-5' exonuclease activity of many DNA polymerases may, therefore, be disadvantageous in situations where one is trying to achieve net synthesis of DNA and/or where fidelity is not of primary concern.

Specification at page 2, lines 14-19.

4. "DNA polymerases lacking 3'-5' exonuclease (proofreading) activity are preferred for applications requiring nucleotide analog incorporation (e.g., DNA sequencing) to prevent removal of nucleotide analogs after incorporation." Specification at page 32, lines 9-11.

5. Archaeal DNA polymerases, such as *Pyrococcus furiosus*, naturally possess 3' to 5' exonuclease (proofreading) activity. Specification at page 24, lines 9-11.

6. It was known in the art that the 3' to 5' exonuclease domain in DNA polymerases comprises three conserved motifs (exo I, exo II, and exo III). Specification at page 32, lines 12-15; *see also* Figure 7B.

7. As stated in the specification, "[t]he 3'-5' exonuclease activity associated with proofreading DNA polymerases can be reduced or abolished by mutagenesis. Sequence comparisons have identified three conserved domains (exo I (DXE), II (NX_{2-3}(F/Y)D), III (YX_3D) in the 3'-5' exonuclease domain of DNA polymerases (reviewed V. Derbyshire, J.K. Pinsonneault, and C.M. Joyce, Methods Enzymol. 262, 363 (1995))." Specification at page 32, lines 12-15.

8. The exo I motif is represented by the consensus amino acid sequence DXE. Specification at page 32, lines 12-15.

9. The exo II motif is represented by the consensus amino acid sequence NX_{2-3}(F/Y)D. Specification at page 32, lines 12-15.

10. The exo III motif is represented by the consensus amino acid sequence YX_3D. Specification at page 32, lines 12-15.

11. It was known in the art that DNA polymerases with 3' to 5' exonuclease activity could be mutated in the conserved exo I, exo II, or exo III motifs to generate mutant DNA polymerases having reduced or abolished 3' to 5' exonuclease activity. Specification at page 32, line 5 to page 33, line 14.

12. There was a known correlation in the art between the conserved exo I, exo II, and exo III motifs of the 3' to 5' exonuclease domain in DNA polymerases and 3' to 5' exonuclease activity. Specification at page 32, line 5 to page 33, line 14.

13. The Examiner does not dispute that there was a known correlation in the art between the conserved exo I, exo II, and exo III motifs of the 3' to 5' exonuclease domain of DNA polymerases and 3' to 5' exonuclease activity.

14. The specification discloses that a wild type Archaeal DNA polymerase or a mutant Archaeal DNA polymerase with deficient 3' to 5' exonuclease activity may be mutated at one or more amino acid positions corresponding to Pro 36, Tyr 37, Ile 38, amino acids 90-97, residues 111-116, and Pro 115 in the wild type Pfu DNA polymerase, and preferably at the valine residue at amino acid position 93 ("V93"). Specification at page 35, line 12 to page 36, line 22.

15. The V93 mutation can confer reduced uracil base detection to the mutant Archaeal DNA polymerase. Specification, page 35, line 1 to page 37, line 2.

16. Wild type Archaeal DNA polymerases stall when they encounter the uracil nucleotide during DNA synthesis. Specification at page 2, lines 20-23.

17. Uracil detection is thought to represent the first step in a pathway to repair DNA cystosine deamination (dCMP → dUMP) in Archaea. Specification at page 2, lines 23-25.

18. Uracil stalling has significant implications for polymerase chain reaction (PCR) amplification with Archaeal DNA polymerases and has been shown to compromise the performance of Archaeal DNA polymerases under standard PCR conditions. Specification, page 2, line 24 to page 3, line 2.

19. Mutant Archaeal DNA polymerases having a mutation at V93 can be useful, for example, in overcoming the problem of uracil stalling. Specification at page 3, lines 22-28.

20. The amino acid sequences represented by SEQ ID NOs. 83-108 correspond to the wild type amino acid sequences of Archaeal DNA polymerases that were known in the art as of

at least the filing date of the present application. Specification at pages 25-32 (Table II) and Figure 7A.

21. The amino acid sequence represented by SEQ ID NO. 83 corresponds to the wild type amino acid sequence of *Thermococcus litoralis* (Vent) DNA polymerase, which was known in the art as of at least the filing date of the present application. Specification at page 25, lines 9-16; and Figure 7A.

22. The amino acid sequence represented by SEQ ID NO. 88 corresponds to the wild type amino acid sequence of *Pyrococcus sp.* (Deep Vent) DNA polymerase, which was known in the art as of at least the filing date of the present application. Specification at page 25, lines 9-11; page 26, line 20 to page 27, line 4; and Figure 7A.

23. The amino acid sequence represented by SEQ ID NO. 89 corresponds to the wild type amino acid sequence of *Pyrococcus furiosus* DNA polymerase, which was known in the art as of at least the filing date of the present application. Specification at page 25, lines 9-11; page 27, lines 6-10; and Figure 7A.

24. The amino acid sequence represented by SEQ ID NO. 90 corresponds to the wild type amino acid sequence of *Thermococcus sp.* (JDF-3) DNA polymerase, which was known in the art as of at least the filing date of the present application. Specification at page 25, lines 9-11; page 27, lines 11-14; and Figure 7A.

25. The amino acid sequence represented by SEQ ID NO. 92 corresponds to the wild type amino acid sequence of *Pyrococcus sp.* (KOD) DNA polymerase, which was known in the art as of at least the filing date of the present application. Specification at page 25, lines 9-11; page 28, lines 1-5; and Figure 7A.

26. The amino acid sequence represented by SEQ ID NO. 93 corresponds to the wild type amino acid sequence of *Thermococcus gorgonarius* (Tgo) DNA polymerase, which was known in the art as of at least the filing date of the present application. Specification at page 25, lines 9-11; page 28, lines 6-11; and Figure 7A.

27. Archaeal DNA polymerases can be divided into 2 classes, 1) Family B/pol I type and 2) pol II type. Specification at page 24, lines 6-9.

28. The specification discloses numerous examples of Archaeal DNA polymerases comprising a mutation at V93, including *Pyrococcus furiosus* (Pfu), *Pyrococcus sp.* (Deep Vent), *Thermococcus gorgonarius* (Tgo), *Pyrococcus sp.* (KOD), *Thermococcus litoralis* (Vent), and *Thermococcus sp.* (JDF-3). Specification at page 36, lines 7-17; page 75, line 21 to page 77, line 16.

29. The specification discloses specific examples of mutant Archaeal DNA polymerases with deficient 3' to 5' exonuclease activity, including a *Thermococcus sp.* (JDF-3) DNA polymerase with a mutation at the position corresponding to D141 and/or E143 in the conserved exo I motif. Specification at page 33, lines 1-7.

30. According to the specification, "one skilled in the art would be able to make an Archaeal DNA polymerase with deficient 3'-5' exonuclease activity by comparing the sequence of the Archaeal DNA polymerase with the sequence of JDF-3 DNA polymerase and by mutating the amino acids within the corresponding conserved exo I, II, or III motifs. In addition, it is also appreciated that one skilled in the art would be able to make an Archaeal DNA polymerase with deficient 3'-5' exonuclease activity by mutating one or more amino acid within the corresponding exo I, II, and III motifs." Specification at page 33, lines 8-14.

31. Example 1 is a prophetic example teaching how to make mutant Archaeal DNA polymerases (Tgo, Pfu, KOD, or JDF-3) comprising a mutation at V93 and at the conserved D141 and E143 residues of the exo I motif and having deficient 3' to 5' exonuclease activity. Specification at page 73, line 8 to page 74, line 16.

32. Example 4 discloses triple mutant Archaeal DNA polymerases (*Pyrococcus furiosus* also known as Pfu) comprising a mutation at V93 (V93R or V93E) and two mutations in the conserved exo I motif (D141A and E143A), where the mutant DNA polymerase possesses deficient 3' to 5' exonuclease activity. Specification at page 78, lines 10-11; Figures 15-16 ("V93R exo").

33. The triple mutant Pfu DNA polymerase is referred to as ("V93R exo") in Figures 15 and 16, with the V93R referring to the mutation at amino acid position 93 and the exo- referring to the deficient 3' to 5' exonuclease activity resulting from the D141A and E143A mutations in the conserved exo I motif (DXE).

34. The Examiner does not provide any evidence or reasoning why the specification does not enable the disclosed mutant Archaeal DNA polymerases comprising 1) a mutation at V93, 2) a mutation in the exo I, exo II, or exo III motif, or 3) a mutation at V93 and at least one mutation in the exo I, exo II, or exo III motif.

35. There was a high level of skill in the art.

36. The Examiner does not contest that there was a high level of skill in the art.

37. Methods for making mutant Archaeal DNA polymerases were known in the art and disclosed in the specification. Specification at pages 41-44 and 48-51.

38. The Examiner acknowledges that "methods to produce specific variants (*i.e.*, exo I, exoII, exoIII and mutants of V93) of a known sequence such as site specific mutagenesis,

random mutagenesis, etc. are well known to the skilled artisan . . . ." Final Office Action at page 8.

39. Methods for assaying DNA polymerases for 3' to 5' exonuclease activity were known in the art and disclosed in the specification. Specification at pages 33-34.

40. The Examiner does not contest that one of skill in the art would be able to test for 3' to 5' exonuclease activity.